cAMP-REGULATED PHOSPHOPROTEIN FOR DIAGNOSTIC AND THERAPEUTIC USE IN NEURODEGENERATIVE DISEASES

The present invention relates to methods of diagnosing, prognosticating and monitoring the progression of neurodegenerative diseases in a subject. Furthermore, methods of therapy control and screening for modulating agents of neurodegenerative diseases are provided. The invention also discloses pharmaceutical compositions, kits, and recombinant animal models.

Neurodegenerative diseases, in particular Alzheimer's disease (AD), have a strongly debilitating impact on a patient's life. Furthermore, these diseases constitute an enormous health, social, and economic burden. AD is the most common neurodegenerative disease, accounting for about 70% of all dementia cases, and it is probably the most devastating age-related neurodegenerative condition affecting about 10% of the population over 65 years of age and up to 45% over age 85 (for a recent review see Vickers et al., *Progress in Neurobiology* 2000, 60: 139-165). Presently, this amounts to an estimated 12 million cases in the US, Europe, and Japan. This situation will inevitably worsen with the demographic increase in the number of old people ("aging of the baby boomers") in developed countries. The neuropathological hallmarks that occur in the brains of individuals with AD are senile plaques, composed of amyloid-β protein, and profound cytoskeletal changes coinciding with the appearance of abnormal filamentous structures and the formation of neurofibrillary tangles.

The amyloid-β (Aβ) protein evolves from the cleavage of the amyloid precursor protein (APP) by different kinds of proteases. The cleavage by the β/γ-secretase leads to the formation of Aβ peptides of different lengths, typically a short more soluble and slow aggregating peptide consisting of 40 amino acids and a longer 42 amino acid peptide, which rapidly aggregates outside the cells, forming the characteristic amyloid plaques (Selkoe, *Physiological Rev* 2001, 81: 741-66; Greenfield et al., *Frontiers Bioscience* 2000, 5: D72-83). Two types of plaques, diffuse plaques and neuritic plaques, can be detected in the brain of AD patients, the latter ones being the classical, most prevalent type. They are primarily found in the cerebral cortex and hippocampus. The neuritic plaques have a diameter of 50µm to 200µm and are composed of insoluble fibrillar amyloids, fragments of dead neurons, of microglia and astrocytes, and other components such as

neurotransmitters, apolipoprotein E, glycosaminoglycans, α1-antichymotrypsin and others. The generation of toxic Aβ deposits in the brain starts very early in the course of AD, and it is discussed to be a key player for the subsequent destructive processes leading to AD pathology. The other pathological hallmarks of AD are neurofibrillary tangles (NFTs) and abnormal neurites, described as neuropil threads (Braak and Braak, *Acta Neuropathol* 1991, 82: 239-259). NFTs emerge inside neurons and consist of chemically altered tau, which forms paired helical filaments twisted around each other. Along the formation of NFTs, a loss of neurons can be observed. It is discussed that said neuron loss may be due to a damaged microtubule-associated transport system (Johnson and Jenkins, *J Alzheimers Dis* 1996, 1: 38-58; Johnson and Hartigan, *J Alzheimers Dis* 1999, 1: 329-351). The appearance of neurofibrillary tangles and their increasing number correlates well with the clinical severity of AD (Schmitt et al., *Neurology* 2000, 55: 370-376).

AD is a progressive disease that is associated with early deficits in memory formation and ultimately leads to the complete erosion of higher cognitive function. The cognitive disturbances include among other things memory impairment, aphasia, agnosia and the loss of executive functioning. A characteristic feature of the pathogenesis of AD is the selective vulnerability of particular brain regions and subpopulations of nerve cells to the degenerative process. Specifically, the temporal lobe region and the hippocampus are affected early and more severely during the progression of the disease. On the other hand, neurons within the frontal cortex, occipital cortex, and the cerebellum remain largely intact and are protected from neurodegeneration (Terry et al., *Annals of Neurology* 1981, 10: 184-92).

The age of onset of AD may vary within a range of 50 years, with early-onset AD occurring in people younger than 65 years of age, and late-onset of AD occurring in those older than 65 years. About 10% of all AD cases suffer from early-onset AD, with only 1-2% being familial, inherited cases.

Currently, there is no cure for AD, nor is there an effective treatment to halt the progression of AD or even to diagnose AD ante-mortem with high probability. Several risk factors have been identified that predispose an individual to develop AD, among them most prominently the epsilon 4 allele of the three different existing alleles (epsilon 2, 3, and 4) of the apolipoprotein E gene (ApoE) (Strittmatter et al., *Proc Natl Acad Sci USA* 1993, 90: 1977-81; Roses, *Ann NY Acad Sci* 1998, 855: 738-43). The polymorphic plasmaprotein ApoE plays a role in the intercellular cholesterol and phospholipid transport by binding low-density lipoprotein receptors,

and it seems to play a role in neurite growth and regeneration. Efforts to detect further susceptibility genes and disease-linked polymorphisms, lead to the assumption that specific regions and genes on human chromosomes 10 and 12 may be associated with late-onset AD (Myers et al., *Science* 2000, 290: 2304-5; Bertram et al., *Science* 2000, 290: 2303; Scott et al., *Am J Hum Genet* 2000, 66: 922-32).

Although there are rare examples of early-onset AD which have been attributed to genetic defects in the genes for amyloid precursor protein (APP) on chromosome 21, presenilin-1 on chromosome 14, and presenilin-2 on chromosome 1, the prevalent form of late-onset sporadic AD is of hitherto unknown etiologic origin. The mutations found to date account for only half of the familial AD cases, which is less than 2% of all AD patients. The late onset and complex pathogenesis of neurodegenerative disorders pose a formidable challenge to the development of therapeutic and diagnostic agents. It is crucial to expand the pool of potential drug targets and diagnostic markers. It is therefore an object of the present invention to provide insight into the pathogenesis of neurological diseases and to provide methods, materials, agents, compositions, and animal models which are suited inter alia for the diagnosis and development of a treatment of these diseases. This object has been solved by the features of the independent claims. The subclaims define preferred embodiments of the present invention.

A group of cAMP-regulated phosphoproteins (ARPPs) has been shown to function as so called intracellular third-messengers in the mammalian central nervous system. Receptor-mediated phosphorylation and dephosphorylation of ARPPs constitute important pathways for the regulation of neuronal functions in response to levels of the important second messenger cAMP and activity of the cAMP-dependent protein kinase, PKA (for recent review, Greengard, Science 2001, 294: 1024-1030). To date, the best characterized ARPPs are DARPP-32 (dopamine and cAMP regulated phosphoprotein of 32 kDa molecular weight), ARPP-16/19 (cAMP regulated phosphoprotein of 16/19 kDa molecular weight), and ARPP-21 (cAMP regulated phosphoprotein of 21 kDa molecular weight), all of which are encoded by separate genes in the human genome. DARPP-32 is encoded on chromosome 17, ARPP-16/19 on chromosome 15, and the ARPP-21 locus is found on chromosome 3 of the human genome. DARPP-32, ARPP-16/19, and ARPP-21 are non-homologous proteins but may have similar or even overlapping functions based on their tissue expression pattern within the human post-mortem brain. Using in situ hybridization

techniques, transcripts for all three ARPPs can be detected in brain regions that receive a rich dopamine innervation from the mesencephalon, i.e. the caudate nucleus, putamen, nucleus accumbens, and the amygdaloid complex. ARPP-16/19, in addition, shows a strong mRNA hybridization signal in the neocortex, whereas DARPP-32 and ARPP-21 showed low levels of signal intensity only (Brene et al., J Neurosci 1994, 14: 985-998). The distribution of ARPP mRNAs overlaps to a large extent with the distribution of the dopamine D1 receptor which thus may regulate the phophorylation status of ARPPs via adenylate cyclase/cAMP and PKA. In fact, the phosphorylation status of DARPP-32 is at the crossroads of multiple complex signaling pathways involving PKA (signaling by receptors for dopamine, opiate, adenosine, serotonin, vasoactive intestinal peptide), the protein phosphatase PP-2B/calcineurin (signaling by receptors for dopamine, gamma-aminobutyric acid, glutamate), and the protein phosphatase PP-1 which controls the state of phosphorylation and activity of numerous physiologically important substrates including neurotransmitter receptors, voltage-gated ion channels, ion pumps, and transcription factors (Greengard, Science 2001, 294: 1024-1030).

A function of ARPP-21 is largely unknown. Human ARPP-21 consists of 89 amino acids and is phophorylated by PKA on serin-56 (Brene et al., J Neurosci 1994, 14: 985-998). The human ARPP-21 isoform cARPP encoding a polypeptide of 89 amino acids has been described in WO00/34477. Available evidence supports the view that ARPP-21 is a cAMP regulated phosphoprotein highly enriched in the cell bodies and terminals of medium-sized spiny neurons of the basal ganglia with the highest levels of immunoreactivity seen in structures comprising the limbic striatum (Ouimet et al., J Neurosci 1989, 9: 865-875). ARPP-21 may therefore play a role as an intracellular third messenger in mediating some of the effects of dopamine, vasoactive intestinal polypeptide, and/or other neurotransmitters acting via cAMP in these dopamine-innervated brain regions (Ouimet et al., J Neurosci 1989, 9: 865-875; Hemmings and Greengard, J Neurosci 1989, 9: 851-864). In fact, the dopamine D1 agonist SKF38393 was shown to increase the state of phosphorylation of ARPP-21 in tissue slices of the substantia nigra of rat brain (Tsou et al., J Neurochem 1993, 60: 1043-1046). Using mouse striatal slices these results were recently corroborated and extended to show that agonists of dopamine D2 receptors cause a strong decrease in ARPP-21 phosphorylation (Caporaso et al., Neuropharmacology 2000, 39: 1637-1644). The likely effector of the dopamine D2 receptor signal is the protein phophatase PP-2A. Several neurological and psychiatric diseases are associated with abnormalities in the dopamine signaling pathways, among them Parkinson's

disease, schizophrenia, attention deficit hyperactivity disorder, and drug abuse. A dysregulation of DARPP-32 function has been postulated to be causally related to the above disorders and, therefore, DARPP-32 can be considered a potential therapeutic target for said diseases (WO 99/20273; US 5777195). A recent study correlates levels of the cAMP regulated phosphoprotein ARPP-19 mRNA and protein in brain tissue from patients suffering from Down syndrome or from Alzheimer's disease (Kim et al., J Neural Transm Suppl 2001, 61: 263-272). Kim and coworkers find normal levels of ARPP-19 mRNA in the temporal lobe and a reduced level of ARPP-19 protein in the cerebellum of AD brain tissue when compared to normal brain.

The present disclosure provides a defined pathophysiological implication and diagnostic and therapeutic utility for a novel and hitherto undescribed human isoform of the cAMP regulated phosphoprotein ARPP-21, herein designated as human TARPP (hTARPP), on the basis of differential expression of hTARPP mRNA in post-mortem brains of patients suffering from Alzheimer's disease in comparison to age-matched healthy individuals. In the mouse, a homologous ARPP-21 splice-variant, called TARPP, encodes a ca. 100 kDa protein that accompanies T cell receptor gene rearrangement and thymocyte education (Kisielow et al., Eur J Immunol 2001, 31: 1141-1149). The name "TARPP" was coined to reflect the thymocyte-specific protein expression in mice. However, murine TARPP mRNA and protein can also be detected in the brain, whereas no mRNA or protein is found in heart, lung, liver, lymph nodes, and spleen. A function for murine TARPP in the brain has not been described.

To date, no experiments have been described that demonstrate a link between the dysregulation of ARPP-21 gene expression and neurodegenerative disorders. Particularly the disclosure in the present invention of the novel human TARPP (hTARPP) isoform, and the identification of a link of this isoform with neurodegenerative diseases, offer new ways, inter alia, for the diagnosis and treatment of neurodegenerative disorders, in particular Alzheimer's disease.

The singular forms "a", "an", and "the" as used herein and in the claims include plural reference unless the context dictates otherwise. For example, "a cell" means as well a plurality of cells, and so forth. The term "and/or" as used in the present specification and in the claims implies that the phrases before and after this term

are to be considered either as alternatives or in combination. For instance, the wording "determination of a level and/or an activity" means that either only a level, or only an activity, or both a level and an activity are determined. The term "level" as used herein is meant to comprise a gage of, or a measure of the amount of, or a concentration of a transcription product, for instance an mRNA, or a translation product, for instance a protein or polypeptide. The term "activity" as used herein shall be understood as a measure for the ability of a transcription product or a translation product to produce a biological effect or a measure for a level of biologically active molecules. The term "activity" also refers to enzymatic activity. The terms "level" and/or "activity" as used herein further refer to gene expression levels or gene activity. Gene expression can be defined as the utilization of the information contained in a gene by transcription and translation leading to the production of a gene product. "Dysregulation" shall mean an upregulation or downregulation of gene expression. A gene product comprises either RNA or protein and is the result of expression of a gene. The amount of a gene product can be used to measure how active a gene is. The term "gene" as used in the present specification and in the claims comprises both coding regions (exons) as well as non-coding regions (e.g. non-coding regulatory elements such as promoters or enhancers, introns, leader and trailer sequences). The term "ORF" is an acronym for "open reading frame" and refers to a nucleic acid sequence that does not possess a stop codon in at least one reading frame and therefore can potentially be translated into a sequence of amino acids. "Regulatory elements" shall comprise inducible and non-inducible promoters, enhancers, operators, and other elements that drive and regulate gene expression. The term "fragment" as used herein is meant to comprise e.g. an alternatively spliced, or truncated, or otherwise cleaved transcription product or translation product. The term "derivative" as used herein refers to a mutant, or an RNA-edited, or a chemically modified, or otherwise altered transcription product, or to a mutant, or chemically modified, or otherwise altered translation product. For instance, a "derivative" may be generated by processes such as altered phosphorylation, or glycosylation, or acetylation, or lipidation, or by altered signal peptide cleavage or other types of maturation cleavage. These processes may occur post-translationally. The term "modulator" as used in the present invention and in the claims refers to a molecule capable of changing or altering the level and/or the activity of a gene, or a transcription product of a gene, or a translation product of a gene. Preferably, a "modulator" is capable of changing or altering the biological activity of a transcription product or a translation product of a gene. Said

modulation, for instance, may be an increase or a decrease in enzyme activity, a change in binding characteristics, or any other change or alteration in the biological, functional, or immunological properties of said translation product of a gene. The terms "agent", "reagent", or "compound" refer to any substance, chemical, composition or extract that have a positive or negative biological effect on a cell, tissue, body fluid, or within the context of any biological system, or any assay system examined. They can be agonists, antagonists, partial agonists or inverse agonists of a target. Such agents, reagents, or compounds may be nucleic acids, natural or synthetic peptides or protein complexes, or fusion proteins. They may also be antibodies, organic or anorganic molecules or compositions, small molecules, drugs and any combinations of any of said agents above. They may be used for testing, for diagnostic or for therapeutic purposes. The terms "oligonucleotide primer" or "primer" refer to short nucleic acid sequences which can anneal to a given target polynucleotide by hybridization of the complementary base pairs and can be extended by a polymerase. They may be chosen to be specific to a particular sequence or they may be randomly selected, e.g. they will prime all possible sequences in a mix. The length of primers used herein may vary from 10 nucleotides to 80 nucleotides. "Probes" are short nucleic acid sequences of the nucleic acid sequences described and disclosed herein or sequences complementary therewith. They may comprise full length sequences, or fragments, derivatives, isoforms, or variants of a given sequence. The identification of hybridization complexes between a "probe" and an assayed sample allows the detection of the presence of other similar sequences within that sample. As used herein, "homolog or homology" is a term used in the art to describe the relatedness of a nucleotide or peptide sequence to another nucleotide or peptide sequence, which is determined by the degree of identity and/or similarity between said sequences compared. The term "variant" as used herein refers to any polypeptide and protein, in reference to polypeptides and proteins disclosed in the present invention, in which one or more amino acids are added and/or substituted and/or deleted and/or inserted at the N-terminus, and/or the C-terminus, and/or within the native amino acid sequences of the native polypeptides or proteins of the present invention. Furthermore, the term "variant" shall include any shorter or longer version of the polypeptides and proteins herein. "Variants" shall also comprise a sequence that has at least about 80% sequence identity, more preferably at least about 90% sequence identity, and most preferably at least about 95% sequence identity with the amino acid sequences of SEQ ID NO. 1. Derivatives, variants and fragments of hTARPP may include, but are not limited to

functional consensus binding motifs for PLCγ and Grb2, as well as an R3H domain or other functional modules within the polypeptide sequence of hTARPP. Variants of a protein molecule shown in SEQ ID NO. 1 include, for example, proteins with conservative amino acid substitutions in highly conservative regions. For example, isoleucine, valine and leucine can each be substituted for one another. Aspartate and glutamate can be substituted for each other. Glutamine and asparagine can be substituted for each other. Serine and threonine can be substituted for each other. Amino acid substitutions in less conservative regions include, for example, isoleucine, valine and leucine, which can each be substituted for one another. Aspartate and glutamate can be substituted for each other. Glutamine and asparagine can be subsituted for each other. Serine and threonine can be substituted for each other. Glycine and alanine can be substituted for each other. Alanine and valine can be substituted for each other. Methionine can be substituted for each of leucine, isoleucine or valine, and vice versa. Lysine and arginine can be substituted for each other. One of aspartate and glutamate can be substituted for one of arginine or lysine, and vice versa. Histidine can be substituted for arginine or lysine, and vice versa. Glutamine and glutamate can be substituted for each other. Asparagine and aspartate can be substituted for each other. Other examples of protein modifications include glycosylation and further post-translational modifications. "Proteins and polypeptides" of the present invention include variants, fragments, and chemical derivatives of the protein comprising SEQ ID NO. 1. As used herein, protein and polypeptide refer to a linear series of amino acid residues connected to one another by peptide bonds between the alpha-amino group and caboxy groups of adjactent amino acid residues. Other covalent bonds, such as amide and disulfide bonds, may also be present. They can include proteins and polypeptides which can be isolated from nature or be produced by recombinant and/or synthetic means. Native proteins or polypeptides refer to naturally-occurring truncated or secreted forms, naturally occurring variant forms (e.g. splice-variants) and naturally occurring allelic variants.

The term "isolated" as used herein is considered to refer to molecules that are removed from their natural environment, i.e. isolated from a cell or from a living organism in which they normally occur, and that are separated or essentially purified from the coexisting components with which they are found to be associated in nature. This notion further means that the sequences encoding such molecules can be linked by the hand of man to polynucleotides, to which they are not linked in their natural state, and that such molecules can be produced by recombinant and/or

synthetic means. Even if for said purposes those sequences may be introduced into living or non-living organisms by methods known to those skilled in the art, and even if those sequences are still present in said organisms, they are still considered to be isolated. In the present invention, the terms "risk", "susceptibility", and "predisposition" are tantamount and are used with respect to the probability of developing a neurodegenerative disease, preferably Alzheimer's disease.

The term 'AD' shall mean Alzheimer's disease. "AD-type neuropathology" as used herein refers to neuropathological, neurophysiological, histopathological and clinical hallmarks as described in the instant invention and as commonly known from state-of-the-art literature (see: Iqbal, Swaab, Winblad and Wisniewski, *Alzheimer`s Disease and Related Disorders (Etiology, Pathogenesis and Therapeutics)*, Wiley & Sons, New York, Weinheim, Toronto, 1999; Scinto and Daffner, *Early Diagnosis of Alzheimer`s Disease*, Humana Press, Totowa, New Jersey, 2000; Mayeux and Christen, *Epidemiology of Alzheimer`s Disease: From Gene to Prevention*, Springer Press, Berlin, Heidelberg, New York, 1999; Younkin, Tanzi and Christen, *Presenilins and Alzheimer`s Disease*, Springer Press, Berlin, Heidelberg, New York, 1998). Neurodegenerative diseases or disorders according to the present invention comprise Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis, Pick's disease, fronto-temporal dementia, progressive nuclear palsy, corticobasal degeneration, cerebro-vascular dementia, multiple system atrophy, argyrophilic grain dementia and other tauopathies, and mild-cognitive impairment. Further conditions involving neurodegenerative processes are, for instance, age-related macular degeneration, narcolepsy, motor neuron diseases, prion diseases, traumatic nerve injury and repair, and multiple sclerosis.

The invention features a novel isolated nucleic acid molecule encoding a protein molecule whose amino acid sequence comprises the sequence shown in SEQ ID NO. 1. Hereinafter, the protein molecule of SEQ ID NO. 1 is denoted human TARPP (hTARPP). Subject to the protein modules of SEQ ID NO. 1, i.e. putative consensus binding motifs for PLCγ and Grb2, as well as an R3H domain, which is a conserved sequence motif, discussed to be involved in the binding of polynucleotides, DNA, single-stranded DNA, and RNA, human TARPP may function as a cAMP regulated protein, as an intracellular third messenger, and/or as a scaffolding protein. Human TARPP may interact with lipids and other proteins, or it may be implicated in nucleic acid binding, in nerve cell signaling pathways, and in organizing and regulating

neuronal function, and thus hTARPP may play a role in neuro-degeneration, in cell protection and regeneration processes. The present invention also features functional variants, derivatives and fragments of hTARPP, which might have a modification of the given primary structure of hTARPP, but whose essential biological function may remain unaffected.

The invention also features the nucleic acid molecules encoding functional variants, or fragments, or derivatives of the protein molecule of SEQ ID NO. 1. Nucleic acid molecules can be DNA molecules, such as genomic DNA molecules or cDNA molecules, or RNA molecules, such as mRNA molecules. In particular, said nucleic acid molecules can be cDNA molecules comprising a nucleotide sequence of SEQ ID NO. 2 or SEQ ID NO. 3.

The invention also features an isolated D N A molecule capable of hybridizing with the complement of the cD N A described in SEQ ID NO. 2 or SEQ ID NO. 3 under stringent conditions. Stringent conditions means that hybridization will be carried out 5°C to 10°C below that temperature at which totally complementary nucleic acids will just hybridize. Optimized stringency conditions for each sequence are established on parameters such as temperature, nucleic acid molecule consistency, salt conditions, and others well known to those of ordinary skill in the art (see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2000). Examples for stringent conditions include (i) 0.2xSSC (standard saline citrate) and 0.1 % SDS at 60 °C and (ii) 50 % formamide, 4xSSC, 50 mM HEPES, pH 7.0, 10x Denhardt's solution, 100 µg/ml thermally denatured salmon sperm DNA at 42 °C. This shall not exclude even higher stringency conditions as mentioned, nor shall it exclude lower stringency conditions as mentioned.

In another aspect, the invention features a vector comprising a nucleic acid encoding a protein molecule shown in SEQ ID NO. 1, or a variant, or derivative, or fragment thereof. In preferred embodiments, a virus, a bacteriophage, or a plasmid comprises the described nucleic acid. In particular, a plasmid adapted for expression in a bacterial cell comprises said nucleic acid molecule, encoding a protein molecule shown in SEQ ID NO. 1, or a fragment, or variant, or derivative thereof, and the regulatory elements necessary for expression of said molecule in a bacterial cell.

In a further aspect, the invention features a plasmid adapted for expression in a yeast cell which comprises a nucleic acid molecule, encoding a protein molecule shown in SEQ ID NO. 1, or a variant, or fragment, or derivative thereof, and the regulatory elements necessary for expression of said molecule in a yeast cell. In another aspect, the invention features a plasmid adapted for expression in a mammalian cell which comprises a nucleic acid molecule, encoding a protein molecule shown in SEQ ID NO. 1, or a fragment, or variant, or derivative thereof, and the regulatory elements necessary for expression of said molecule in a mammalian cell.

In a further aspect, the invention features a cell comprising a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, or a fragment, or derivative, or a variant thereof. The present invention also features cells comprising a DNA molecule capable of hybridizing with the complement of the c D N A described in SEQ ID NO. 2 or SEQ ID NO. 3 under stringent conditions. In preferred embodiments, said cell is a bacterial cell, a yeast cell, a mammalian cell, or a cell of an insect. In particular, the invention features a bacterial cell comprising a plasmid adapted for expression in a bacterial cell, said plasmid comprising a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, or a fragment, or a derivative, or a variant thereof, and the regulatory elements necessary for expression of said molecule in the bacterial cell. The invention also features a yeast cell comprising a plasmid adapted for expression in a yeast cell, said plasmid comprises a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, or a fragment, or a derivative, or a variant thereof, and the regulatory elements necessary for expression of said molecule in the yeast cell. The invention further features a mammalian cell comprising a plasmid adapted for expression in a mammalian cell, said plasmid comprising a nucleic acid molecule encoding a protein molecule shown in SEQ ID NO. 1, or a variant, or a derivative, or a fragment thereof, and the regulatory elements necessary for expression of said molecule in the mammalian cell.

In one aspect the present invention features a protein molecule shown in SEQ ID NO. 1. Furthermore, the present invention features a protein molecules shown in SEQ ID NO. 1, or a fragment, or derivative, or variant thereof, for use as a

diagnostic target for detecting a neurodegenerative disease, preferably Alzheimer's disease.

The present invention further features a protein molecule shown in SEQ ID NO. 1, or a fragment, or derivative, or variant thereof, for use as a screening target for reagents or compounds preventing, or treating, or ameliorating a neurodegenerative disease, preferably Alzheimer's disease.

The invention further features an antibody specifically immunoreactive with an immunogen, wherein said immunogen is a translation product of the human TARPP gene shown in SEQ ID NO. 1, or a fragment, or a variant, or a derivative thereof. The immunogen may comprise immunogenic or antigenic epitopes or portions of a translation product of said gene, wherein said immunogenic or antigenic portion of a translation product is a polypeptide, and wherein said polypeptide elicits an antibody response in an animal, and wherein said polypeptide is immunospecifically bound by said antibody. Methods for generating antibodies are well known in the art (see Harlow et al., *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1988). The term "antibody", as employed in the present invention, encompasses all forms of antibodies known in the art, such as polyclonal, monoclonal, chimeric, recombinatorial, anti-idiotypic, humanized, or single chain antibodies, as well as fragments thereof (see Dubel and Breitling, *Recombinant Antibodies*, Wiley-Liss, New York, NY, 1999). Antibodies of the present invention are useful, for instance, in a variety of diagnostic and therapeutic methods, based on state-in-the-art techniques (see Harlow and Lane, *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999 and Edwards R., *Immunodiagnostics: A Practical Approach*, Oxford University Press, Oxford, England, 1999) such as enzyme-immuno assays (e.g. enzyme-linked immunosorbent assay, ELISA), radioimmuno assays, chemoluminescence-immuno assays, Western-blot, immunoprecipitation and antibody microarrays. These methods involve the detection of translation products of the human TARPP gene.

In a preferred embodiment of the present invention, said antibodies can be used for detecting the pathological state of a cell in a sample from a subject, comprising immunocytochemical staining of said cell with said antibody, wherein an altered degree of staining, or an altered staining pattern in said cell compared to a cell

representing a known health status indicates a pathological state of said cell. The invention is particularly suited to detect pathological structures in the brain of a subject. It is also especially suited to detect pathological cells of the muscular system, prostate, stomach, testis, ovary, adrenal glands, mammary glands, liver, spleen, lung, trachea or placenta. Preferably, the pathological state relates to a neurodegenerative disease, in particular to Alzheimer's disease. Immunocytochemical staining of a cell can be carried out by a number of different experimental methods well known in the art. It might be preferred, however, to apply an automated method for the detection of antibody binding, wherein the determination of the degree of staining of a cell, or the determination of the cellular or subcellular staining pattern of a cell, or the topological distribution of an antigen on the cell surface or among organelles and other subcellular structures within the cell, are carried out according to the method described in US patent 6150173.

In one aspect, the invention features a method of diagnosing or prognosticating a neurodegenerative disease in a subject, or determining whether a subject is at increased risk of developing said disease. The method comprises: determining a level, or an activity, or both said level and said activity of (i) a transcription product of the gene coding for hTARPP, and/or of (ii) a translation product of the gene coding for hTARPP, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample from said subject and comparing said level, and/or said activity to a reference value representing a known disease or health status, thereby diagnosing or prognosticating said neurodegenerative disease in said subject, or determining whether said subject is at increased risk of developing said neurodegenerative disease.

The invention also relates to the construction and the use of primers and probes which are unique to the nucleic acid sequences, or fragments, or variants thereof, as disclosed in the present invention. The oligonucleotide primers and/or probes can be labeled specifically with fluorescent, bioluminescent, magnetic, or radioactive substances. The invention further relates to the detection and the production of said nucleic acid sequences, or fragments and/or variants thereof, using said specific oligonucleotide primers in appropriate combinations. PCR-analysis, a method well known to those skilled in the art, can be performed with said primer combinations to amplify said gene specific nucleic acid sequences from a sample containing nucleic acids. Such sample may be derived either from healthy or diseased subjects.

Whether an amplification results in a specific nucleic acid product or not, and whether a fragment of different length can be obtained or not, may be indicative for a neurodegenerative disease, in particular Alzheimer's disease. Thus, the invention provides nucleic acid sequences, oligonucleotide primers, and probes of at least 10 bases in length up to the entire coding and gene sequences, useful for the detection of gene mutations and single nucleotide polymorphisms in a given sample comprising nucleic acid sequences to be examined, which may be associated with neurodegenerative diseases, in particular Alzheimer's disease. This feature has utility for developing rapid DNA-based diagnostic tests, preferably also in the format of a kit.

In a further aspect, the invention features a method of monitoring the progression of a neurodegenerative disease in a subject. A level, or an activity, or both said level and said activity, of (i) a transcription product of the gene coding for hTARPP, and/or of (ii) a translation product of the gene coding for hTARPP, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample from said subject is determined. Said level and/or said activity is compared to a reference value representing a known disease or health status. Thereby the progression of said neurodegenerative disease in said subject is monitored.

In still a further aspect, the invention features a method of evaluating a treatment for a neurodegenerative disease, comprising determining a level, or an activity, or both said level and said activity of (i) a transcription product of the gene coding for hTARPP, and/or of (ii) a translation product of the gene coding for hTARPP, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample obtained from a subject being treated for said disease. Said level, or said activity, or both said level and said activity are compared to a reference value representing a known disease or health status, thereby evaluating the treatment for said neurodegenerative disease.

In a further preferred embodiment of the herein claimed methods, kits, recombinant animals, molecules, assays, and uses of the instant invention, said neurodegenerative disease or disorder is Alzheimer's disease, and said subjects suffer from Alzheimer's disease.

The present invention discloses the differential expression and regulation of hTARPP in specific brain regions of Alzheimer's disease patients. Consequently, the gene coding for hTARPP and its corresponding translation products may have a causative role in the regional selective neuronal degeneration typically observed in Alzheimer's disease. Alternatively, hTARPP may confer a neuroprotective function to the remaining surviving nerve cells. Based on these disclosures, the present invention has utility for the diagnostic evaluation and prognosis as well as for the identification of a predisposition to a neurodegenerative disease, in particular Alzheimer's disease. Furthermore, the present invention provides methods for the diagnostic monitoring of patients undergoing treatment for such a disease.

It is preferred that the sample to be analyzed and determined is selected from the group comprising brain tissue, or other tissues, or other body cells. The sample can also comprise cerebrospinal fluid or other body fluids including saliva, urine, blood, serum plasma, or mucus. Preferably, the methods of diagnosis, prognosis, monitoring the progression or evaluating a treatment for a neurodegenerative disease, according to the instant invention, can be practiced *ex corpore*, and such methods preferably relate to samples, for instance, body fluids or cells, removed, collected, or isolated from a subject or patient.

In further preferred embodiments, said reference value is that of a level, or an activity, or both said level and said activity of (i) a transcription product of the gene coding for hTARPP, and/or of (ii) a translation product of the gene coding for hTARPP, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a sample from a subject not suffering from said neurodegenerative disease.

In preferred embodiments, an alteration in the level and/or activity of a transcription product of the gene coding for human TARPP and/or a translation product of the gene coding for human TARPP protein in a sample cell, or tissue, or body fluid from said subject relative to a reference value representing a known health status indicates a diagnosis, or prognosis, or increased risk of becoming diseased with a neurodegenerative disease, particularly Alzheimer's disease.

In preferred embodiments, measurement of the level of transcription products of the gene coding for hTARPP is performed in a sample from a subject using a

quantitative PCR-analysis with primer combinations to amplify said gene specific sequences from cDNA obtained by reverse transcription of RNA extracted from a sample of a subject. A Northern blot with probes specific for said gene can also be applied. It might further be preferred to measure transcription products by means of chip-based microarray technologies. These techniques are known to those of ordinary skill in the art (see Sambrook and Russell, *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001; Schena M., *Microarray Biochip Technology*, Eaton Publishing, Natick, MA, 2000). An example of an immunoassay is the detection and measurement of enzyme activity as disclosed and described in the patent application WO 02/14543.

Furthermore, a level and/or an activity of a translation product of the gene coding for hTARPP, and/or a fragment, or derivative, or variant of said translation product, and/or the level of activity of said translation product of the gene coding for hTARPP, and/or a fragment, or derivative, or variant thereof, can be detected using an immunoassay, an activity assay, and/or a binding assay. These assays can measure the amount of binding between said protein molecule and an anti-protein antibody by the use of enzymatic, chromodynamic, radioactive, magnetic, or luminescent labels which are attached to either the anti-protein antibody or a secondary antibody which binds the anti-protein antibody. In addition, other high affinity ligands may be used. Immunoassays which can be used include e.g. ELISAs, Western blots and other techniques known to those of ordinary skill in the art (see Harlow and Lane, *Using Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1999 and Edwards R, *Immunodiagnostics: A Practical Approach*, Oxford University Press, Oxford; England, 1999). All these detection techniques may also be employed in the format of microarrays, protein-arrays, antibody microarrays, tissue microarrays, electronic biochip or protein-chip based technologies (see Schena M., *Microarray Biochip Technology*, Eaton Publishing, Natick, MA, 2000).

In a preferred embodiment, the level, or the activity, or both said level and said activity of (i) a transcription product of the gene coding for hTARPP, and/or of (ii) a translation product of the gene coding for hTARPP, and/or of (iii) a fragment, or derivative, or variant of said transcription or translation product in a series of samples taken from said subject over a period of time is compared, in order to monitor the progression of said disease. In further preferred embodiments, said

subject receives a treatment prior to one or more of said sample gatherings. In yet another preferred embodiment, said level and/or activity is determined before and after said treatment of said subject.

In another aspect, the invention features a kit for diagnosing or prognosticating neurodegenerative diseases, in particular Alzheimer's disease, or determining the propensity or predisposition of a subject to develop a neurodegenerative disease, in particular Alzheimer's disease, said kit comprising:

(a) at least one reagent which is selected from the group consisting of (i) reagents that selectively detect a transcription product of the gene coding for hTARPP, (ii) reagents that selectively detect a translation product of the gene coding for hTARPP; and

(b) instruction for diagnosing, or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of a subject to develop such a disease by

- detecting a level, or an activity, or both said level and said activity, of said transcription product and/or said translation product of the gene coding for hTARPP, in a sample from said subject; and
- diagnosing or prognosticating a neurodegenerative disease, in particular Alzheimer's disease, or determining the propensity or predisposition of said subject to develop such a disease,

wherein a varied level, or activity, or both said level and said activity, of said transcription product and/or said translation product compared to a reference value representing a known health status; or a level, or activity, or both said level and said activity, of said transcription product and/or said translation product similar or equal to a reference value representing a known disease status, indicates a diagnosis or prognosis of a neurodegenerative disease, in particular Alzheimer's disease, or an increased propensity or predisposition of developing such a disease. The kit, according to the present invention, may be particularly useful for the identification of individuals that are at risk of developing a neurodegenerative disease, in particular Alzheimer's disease. Consequently, the kit, according to the invention, may serve as a means for targeting identified individuals for early preventive measures or therapeutic intervention prior to disease onset, before irreversible damage in the course of the disease has been inflicted. Furthermore, in preferred embodiments, the kit featured in the invention is useful for monitoring a progression of a neurodegenerative disease, in particular Alzheimer's disease, in a subject, as well

as monitoring success or failure of therapeutic treatment for such a disease of said subject.

In another aspect, the invention features a method of treating or preventing a neurodegenerative disease, in particular Alzheimer's disease, in a subject comprising the administration to said subject in a therapeutically or prophylactically effective amount of an agent or agents which directly or indirectly affect a level, or an activity, or both said level and said activity, of (i) the gene coding for hTARPP, and/or (ii) a transcription product of the gene coding for hTARPP, and/or (iii) a translation product of the gene coding for hTARPP, and/or (iv) a fragment, or derivative, or variant of (i) to (iii). Said agent may comprise a small molecule, or it may also comprise a peptide, an oligopeptide, or a polypeptide. Said peptide, oligopeptide, or polypeptide may comprise an amino acid sequence shown in SEQ ID NO. 1, or a fragment, or derivative, or a variant thereof. An agent for treating or preventing a neurodegenerative disease, in particular AD, according to the instant invention, may also consist of a nucleotide, an oligonucleotide, or a polynucleotide. Said oligonucleotide or polynucleotide may comprise a nucleotide sequence of the gene coding for hTARPP shown in SEQ ID NO. 2 or SEQ ID NO. 3, either in sense orientation or in antisense orientation.

In preferred embodiments, the method comprises the application of per se known methods of gene therapy and/or antisense nucleic acid technology to administer said agent or agents. In general, gene therapy includes several approaches: molecular replacement of a mutated gene, addition of a new gene resulting in the synthesis of a therapeutic protein, and modulation of endogenous cellular gene expression by recombinant expression methods or by drugs. Gene-transfer techniques are described in detail (see e.g. Behr, *Acc Chem Res* 1993, 26: 274-278 and Mulligan, *Science* 1993, 260: 926-931) and include direct gene-transfer techniques such as mechanical microinjection of DNA into a cell as well as indirect techniques employing biological vectors (like recombinant viruses, especially retroviruses) or model liposomes, or techniques based on transfection with DNA coprecipitation with polycations, cell membrane pertubation by chemical (solvents, detergents, polymers, enzymes) or physical means (mechanic, osmotic, thermic, electric shocks). The postnatal gene transfer into the central nervous system has been described in detail (see e.g. Wolff, *Curr Opin Neurobiol* 1993, 3: 743-748).

In particular, the invention features a method of treating or preventing a neurodegenerative disease by means of antisense nucleic acid therapy, i.e. the down-regulation of an inappropriately expressed or defective gene by the introduction of antisense nucleic acids or derivatives thereof into certain critical cells (see e.g. Gillespie, *DN&P* 1992, 5: 389-395; Agrawal and Akhtar, *Trends Biotechnol* 1995, 13: 197-199; Crooke, *Biotechnology* 1992, 10: 882-6). Apart from hybridization strategies, the application of ribozymes, i.e. RNA molecules that act as enzymes, destroying RNA that carries the message of disease has also been described (see e.g. Barinaga, *Science* 1993, 262: 1512-1514). In preferred embodiments, the subject to be treated is a human, and therapeutic antisense nucleic acids or derivatives thereof are directed against hTARPP. It is preferred that cells of the central nervous system, preferably the brain, of a subject are treated in such a way. Cell penetration can be performed by known strategies such as coupling of antisense nucleic acids and derivatives thereof to carrier particles, or the above described techniques. Strategies for administering targeted therapeutic oligodeoxynucleotides are known to those of skill in the art (see e.g. Wickstrom, *Trends Biotechnol* 1992, 10: 281-287). In some cases, delivery can be performed by mere topical application. Further approaches are directed to intracellular expression of antisense RNA. In this strategy, cells are transformed *ex vivo* with a recombinant gene that directs the synthesis of an RNA that is complementary to a region of target nucleic acid. Therapeutical use of intracellularly expressed antisense RNA is procedurally similar to gene therapy. A recently developed method of regulating the intracellular expression of genes by the use of double-stranded RNA, known variously as RNA interference (RNAi), can be another effective approach for nucleic acid therapy (Hannon, *Nature* 2002, 418: 244-251).

In further preferred embodiments, the method comprises grafting donor cells into the central nervous system, preferably the brain, of said subject, or donor cells preferably treated so as to minimize or reduce graft rejection, wherein said donor cells are genetically modified by insertion of at least one transgene encoding said agent or agents. Said transgene might be carried by a viral vector, in particular a retroviral vector. The transgene can be inserted into the donor cells by a nonviral physical transfection of DNA encoding a transgene, in particular by microinjection. Insertion of the transgene can also be performed by electroporation, chemically mediated transfection, in particular calcium phosphate transfection, and liposomal

mediated transfection (see Mc Celland and Pardee, *Expression Genetics: Accelerated and High-Throughput Methods*, Eaton Publishing, Natick, MA, 1999).

In preferred embodiments, said agent for treating and preventing a neurodegenerative disease, in particular Alzheimer's disease, is a therapeutic protein which can be administered to said subject, preferably a human, by a process comprising introducing subject cells into said subject, said subject cells having been treated *in vitro* to insert a DNA segment encoding said therapeutic protein, said subject cells expressing *in vivo* in said subject a therapeutically effective amount of said therapeutic protein. Said DNA segment can be inserted into said cells *in vitro* by a viral vector, in particular a retroviral vector.

Methods of treatment, according to the present invention, comprise the application of therapeutic cloning, transplantation, and stem cell therapy using embryonic stem cells or embryonic germ cells and neuronal adult stem cells, combined with any of the previously described cell- and gene therapeutic methods. Stem cells may be totipotent or pluripotent. They may also be organ-specific. Strategies for repairing diseased and/or damaged brain cells or tissue comprise (i) taking donor cells from an adult tissue. Nuclei of those cells are transplanted into unfertilized egg cells from which the genetic material has been removed. Embryonic stem cells are isolated from the blastocyst stage of the cells which underwent somatic cell nuclear transfer. Use of differentiation factors then leads to a directed development of the stem cells to specialized cell types, preferably neuronal cells (Lanza et al., *Nature Medicine* 1999, 9: 975-977), or (ii) purifying adult stem cells, isolated from the central nervous system, or from bone marrow (mesenchymal stem cells), for in vitro expansion and subsequent grafting and transplantation, or (iii) directly inducing endogenous neural stem cells to proliferate, migrate, and differentiate into functional neurons (Peterson DA, *Curr. Opin. Pharmacol.* 2002, 2: 34-42). Adult neural stem cells are of great potential for repairing damaged or diseased brain tissues, as the germinal centers of the adult brain are basically free of neuronal damage or dysfunction (Colman A, *Drug Discovery World* 2001, 7: 66-71).

In preferred embodiments, the subject for treatment or prevention, according to the present invention, can be a human, an experimental animal, e.g. a mouse or a rat, a domestic animal, or a non-human primate. The experimental animal can be an

animal model for a neurodegenerative disorder, e.g. a transgenic mouse and/or a knock-out mouse with an Alzheimer's-type neuropathology.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) a gene coding for hTARPP, and/or (ii) a transcription product of the gene coding for hTARPP and/or (iii) a translation product of the gene coding for hTARPP, and/or (iv) a fragment, or derivative, or variant of (i) to (iii).

In an additional aspect, the invention features a pharmaceutical composition comprising said modulator and preferably a pharmaceutical carrier. Said carrier refers to a diluent, adjuvant, excipient, or vehicle with which the modulator is administered.

In a further aspect, the invention features a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) a gene coding for hTARPP, and/or (ii) a transcription product of the gene coding for hTARPP, and/or (iii) a translation product of the gene coding for hTARPP, and/or (iv) a fragment, or derivative, or variant of (i) to (iii) for use in a pharmaceutical composition.

In another aspect, the invention provides for the use of a modulator of an activity, or a level, or both said activity and said level of at least one substance which is selected from the group consisting of (i) a gene coding for hTARPP, and/or (ii) a transcription product of the gene coding for hTARPP and/or (iii) a translation product of the gene coding for hTARPP, and/or (iv) a fragment, or derivative, or variant of (i) to (iii) for a preparation of a medicament for treating or preventing a neurodegenerative disease, in particular Alzheimer's disease.

In one aspect, the present invention also provides a kit comprising one or more containers filled with a therapeutically or prophylactically effective amount of said pharmaceutical composition.

In a further aspect, the invention features a recombinant, non-human animal comprising a non-native gene sequence coding for hTARPP, or a fragment, or a variant, or a derivative thereof. The generation of said recombinant, non-human

animal comprises (i) providing a gene targeting construct containing said gene sequence and a selectable marker sequence, and (ii) introducing said targeting construct into a stem cell of a non-human animal, and (iii) introducing said non-human animal stem cell into a non-human embryo, and (iv) transplanting said embryo into a pseudopregnant non-human animal, and (v) allowing said embryo to develop to term, and (vi) identifying a genetically altered non-human animal whose genome comprises a modification of said gene sequence in both alleles, and (vii) breeding the genetically altered non-human animal of step (vi) to obtain a genetically altered non-human animal whose genome comprises a modification of said endogenous gene, wherein said gene is mis-expressed, or under-expressed, or over-expressed, and wherein said disruption or alteration results in said non-human animal exhibiting a predisposition to developing symptoms of neuropathology similar to a neurodegenerative disease, in particular Alzheimer's disease. Strategies and techniques for the generation and construction of such an animal are known to those of ordinary skill in the art (see e.g. Capecchi, *Science* 1989, 244: 1288-1292 and Hogan et al., 1994, *Manipulating the Mouse Embryo: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York and Jackson and Abbott, *Mouse Genetics and Transgenics: A Practical Approach*, Oxford University Press, Oxford, England, 1999). It is preferred to make use of such a recombinant non-human animal as an animal model for investigating neurodegenerative diseases, in particular Alzheimer's disease. Such an animal may be useful for screening, testing and validating compounds, agents and modulators in the development of diagnostics and therapeutics to treat neurodegenerative diseases, in particular Alzheimer's disease.

In another aspect, the invention features an assay for screening for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases and disorders of one or more substances selected from the group consisting of (i) a gene coding for hTARPP, and/or (ii) a transcription product of the gene coding for hTARPP, and/or (iii) a translation product of the gene coding for hTARPP, and/or (iv) a fragment, or derivative, or variant of (i) to (iii). This screening method comprises (a) contacting a cell with a test compound, and (b) measuring the activity, or the level, or both the activity and the level of one or more substances recited in (i) to (iv), and (c) measuring the activity, or the level, or both the activity and the level of said substances in a control cell not contacted with said test compound, and (d) comparing the levels of the substance in the cells of step (b) and (c), wherein an

alteration in the activity and/or level of said substances in the contacted cells indicates that the test compound is a modulator of said diseases and disorders.

In one further aspect, the invention features a screening assay for a modulator of neurodegenerative diseases, in particular Alzheimer's disease, or related diseases and disorders of one or more substances selected from the group consisting of (i) a gene coding for hTARPP, and/or (ii) a transcription product of the gene coding for hTARPP, and/or (iii) a translation product of the gene coding for hTARPP, and/or (iv) a fragment, or derivative, or variant of (i) to (iii), comprising (a) administering a test compound to a test animal which is predisposed to developing or has already developed symptoms of a neurodegenerative disease or related diseases or disorders, and (b) measuring the activity and/or level of one or more substances recited in (i) to (iv), and (c) measuring the activity and/or level of said substances in a matched control animal which is equally predisposed to developing or has already developed symptoms of said diseases and to which animal no such test compound has been administered, and (d) comparing the activity and/or level of the substance in the animals of step (b) and (c), wherein an alteration in the activity and/or level of substances in the test animal indicates that the test compound is a modulator of said diseases and disorders.

In a preferred embodiment, said test animal and/or said control animal is a recombinant non-human animal which expresses hTARPP, or a fragment, or a variant, or a derivative thereof, under the control of a transcriptional regulatory element which is not the native hTARPP gene transcriptional control regulatory element.

In another embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a modulator of neurodegenerative diseases by a method of the aforementioned screening assays and (ii) admixing the modulator with a pharmaceutical carrier. However, said modulator may also be identifiable by other types of screening assays.

In another aspect, the present invention provides for an assay for testing a compound, preferably for screening a plurality of compounds, for inhibition of binding between a ligand and hTARPP, or a fragment, or derivative, or variant thereof. Said screening assay comprises the steps of (i) adding a liquid suspension

of said hTARPP, or a fragment, or variant, or derivative thereof, to a plurality of containers, and (ii) adding a compound or a plurality of compounds to be screened for said inhibition to said plurality of containers, and (iii) adding fluorescently labelled ligand to said containers, and (iv) incubating said hTARPP, or said fragment, or variant, or derivative thereof, and said compound or plurality of compounds, and said fluorescently labelled ligand, and (v) measuring the amounts of fluorescence associated with said hTARPP, or with said fragment, or variant, or derivative thereof, and (vi) determining the degree of inhibition by one or more of said compounds of binding of said ligand to said hTARPP, or said fragment, or variant, or derivative thereof. Instead of utilizing a fluorescently labelled ligand, it might in some aspects be preferred to use any other detectable label known to the person skilled in the art, e.g. radioactive labels, and detect it accordingly. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to inhibit the binding of a ligand to a gene product of the gene coding for hTARPP, or a fragment, or variant, or derivative thereof. One example of a fluorescent binding assay, in this case based on the use of carrier particles, is disclosed and described in patent application WO 00/52451. A further example is the competitive assay method as described in patent WO 02/01226. Preferred signal detection methods for screening assays of the instant invention are described in the following patent applications: WO 96/13744, WO 98/16814, WO 98/23942, WO 99/17086, WO 99/34195, WO 00/66985, WO 01/59436, WO 01/59416.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as an inhibitor of binding between a ligand and a gene product of the gene coding for hTARPP by the aforementioned inhibitory binding assay and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another aspect, the invention features an assay for testing a compound, preferably for screening a plurality of compounds to determine the degree of binding of said compounds to hTARPP, or to a fragment, a variant, or a derivative thereof. Said screening assay comprises (i) adding a liquid suspension of said hTARPP, or a fragment, or variant, or derivative thereof, to a plurality of containers, and (ii) adding a fluorescently labelled compound or a plurality of fluorescently labelled compounds

to be screened for said binding to said plurality of containers, and (iii) incubating said hTARPP, or said fragment, or variant, or derivative thereof, and said fluorescently labelled compound or fluorescently labelled compounds, and (iv) measuring the amounts of fluorescence associated with said hTARPP, or with said fragment, or variant, or derivative thereof, and (v) determining the degree of binding by one or more of said compounds to said hTARPP, or said fragment, or variant, or derivative thereof. In this type of assay it might be preferred to use a fluorescent label. However, any other type of detectable label might also be employed. Said method may be useful for the identification of novel compounds as well as for evaluating compounds which have been improved or otherwise optimized in their ability to bind to hTARPP.

In one further embodiment, the present invention provides a method for producing a medicament comprising the steps of (i) identifying a compound as a binder to a gene product of the gene coding for hTARPP by the aforementioned binding assays and (ii) admixing the compound with a pharmaceutical carrier. However, said compound may also be identifiable by other types of screening assays.

In another embodiment, the present invention provides for a medicament obtainable by any of the methods according to the herein claimed screening assays. In one further embodiment, the instant invention provides for a medicament obtained by any of the methods according to the herein claimed screening assays.

Other features and advantages of the invention will be apparent from the following description of figures and examples which are illustrative only and not intended to limit the remainder of the disclosure in any way.

Figure 1 depicts the brain regions with selective vulnerability to neuronal loss and degeneration in Alzheimer's disease. Primarily, neurons within the inferior temporal lobe, the entorhinal cortex, the hippocampus, and the amygdala are subject to degenerative processes in Alzheimer's disease (Terry et al., *Annals of Neurology* 1981, 10:184-192). These brain regions are mostly involved in the processing of learning and memory functions. In contrast, neurons within the frontal cortex, the occipital cortex, and the cerebellum remain largely intact and preserved from neurodegenerative processes in Alzheimer's disease. Brain tissues from the frontal

cortex (F), the temporal cortex (T), and the hippocampus (H) of Alzheimer's disease patients and healthy, age-matched control individuals were used for the herein disclosed examples. For illustrative purposes, the image of a normal healthy brain was taken from a publication by Strange (*Brain Biochemistry and Brain Disorders*, Oxford University Press, Oxford, 1992, p.4).

Figure 2 and 3 illustrate the verification of the differential expression of the human TARPP (hTARPP) gene in AD brain tissues by quantitative RT-PCR analysis. Quantification of RT-PCR products from RNA samples collected from the frontal cortex (F) and the temporal cortex (T) of AD patients (Figure 2a) and samples from the frontal cortex (F) and the hippocampus (H) of AD patients (Figure 3a) was performed by the LightCycler rapid thermal cycling technique. Likewise, samples of healthy, age-matched control individuals were compared (Figure 2b for frontal cortex and temporal cortex, Figure 3b for frontal cortex and hippocampus). The data were normalized to the combined average values of a set of standard genes which showed no significant differences in their gene expression levels. Said set of standard genes consisted of genes for cyclophilin B, the ribosomal protein S9, the transferrin receptor, GAPDH, and beta-actin. The figure depicts the kinetics of amplification by plotting the cycle number against the amount of amplified material as measured by its fluorescence. Note that the amplification kinetics of hTARPP cDNAs from both, the frontal and temporal cortices of a normal control individual, and from the frontal cortex and hippocampus of a normal control individual, respectively, during the exponential phase of the reaction are juxtaposed (Figures 2b and 3b, arrowheads), whereas in Alzheimer's disease (Figures 2a and 3a, arrowheads) there is a significant separation of the corresponding curves, indicating a differential expression of the human TARPP gene in the respective analyzed brain regions.

Figure 4 discloses the protein sequence of human TARPP (hTARPP); SEQ ID NO. 1. The full length human TARPP protein consists of 813 amino acids.

Figure 5 shows an alignment of the amino acid sequence of SEQ ID NO.1, hTARPP protein, with mouse (*Mus musculus*) TARPP amino acid sequence (GenBank accession number af324451).

Figure 6 represents the nucleotide sequence of SEQ ID NO. 2, the coding sequence of the human TARPP gene, comprising 2442 nucleotides.

Figure 7 shows the nucleotide sequence of SEQ ID NO. 3, the hTARPP cDNA, comprising 3212 nucleotides. Primers used for quantitative PCR analysis are located from nucleotide 2471 to 2493 for the forward primer and from nucleotide 2518 to 2539 for the reverse primer.

Figure 8 depicts SEQ ID NO. 4, the nucleotide sequence of the 69 bp cDNA fragment, amplified with the primers used for quantitative PCR analysis. For the location of the primers refer to SEQ ID NO. 3, Figure 7.

Figure 9 charts the schematic alignment of SEQ ID NO. 4, the hTARPP cDNA fragment, SEQ ID NO. 2, the coding sequence of the hTARPP gene, and SEQ ID NO. 3, the hTARPP gene nucleotide sequence derived from the alignment of human EST nucleotide sequences found in the GenBank genetic sequence database. EST numbers are written on the left side, all sequences are 5' to 3' directed.

Figure 10 depicts human cerebral cortex labeled with an affinity-purified rabbit anti-hTARPP antiserum raised against a peptide corresponding to amino acids 566-580 (green signals). Strong immunoreactivity of human TARPP was detected in both, pre-central cortex (CT) and white matter (WM) (Figure 10a, low magnification). In the cortex, hTARPP is mainly detected in the cytoplasm of neuronal cell bodies and in some distal segments of neuronal processes (Figure 10b, high magnification). Moreover, axonal filaments and the cytoplasm of some glia cells were immuno-positive in the white matter. The same immunostaining pattern was observed by using another antiserum raised against a peptide mapping to amino acids 325-341 of hTARPP. Blue signals indicate nuclei stained with DAPI.

Table 1 lists expression levels in the frontal cortex relative to the temporal cortex for the transcription product of the hTARPP gene in seven Alzheimer's disease patients, herein identified by internal reference numbers P010, P011, P012, P014, P016, P017, P019 (1.34 to 4.11 fold) and five healthy, age-matched control individuals, herein identified by internal reference numbers C005, C008, C011, C012, C014 (0.47 to 1.39 fold). The values shown are reciprocal values according to the formula described herein (see below).

Table 2 lists the hTARPP gene expression levels in the frontal cortex relative to the hippocampus in six Alzheimer's disease patients, herein identified by internal reference numbers P010, P011, P012, P014, P016, P019 (1.21 to 5.51 fold) and three healthy, age-matched control individuals, herein identified by internal reference numbers C004, C005, C008 (1.10 to 1.76 fold). The values shown are reciprocal values according to the formula described herein (see below).

EXAMPLE I:

(i) Brain tissue dissection from patients with Alzheimer's disease:

Brain tissues from Alzheimer's disease patients and age-matched control subjects were collected within 6 hours post-mortem and immediately frozen on dry ice. Sample sections from each tissue were fixed in paraformaldehyde for histopathological confirmation of the diagnosis. Brain areas for differential expression analysis were identified (see Figure 1) and stored at – 80 °C until RNA extractions were performed.

(ii) Isolation of total mRNA:

Total RNA was extracted from post-mortem brain tissue by using the RNeasy kit (Qiagen) according to the manufacturer's protocol. The accurate RNA concentration and the RNA quality was determined with the DNA LabChip system using the Agilent 2100 Bioanalyzer (Agilent Technologies). For additional quality testing of the prepared RNA, i.e. exclusion of partial degradation and testing for DNA contamination, specifically designed intronic GAPDH oligonucleotides and genomic DNA as reference control were used to generate a melting curve with the LightCycler technology as described in the manufacturer's protocol (Roche).

(iii) Determination of differential expression by quantitative RT-PCR:

In order to identify changes in gene expression in different tissues we examined differential expression of the hTARPP gene using the LightCycler technology (Roche). This technique features rapid thermal cyling for the polymerase chain reaction as well as real-time measurement of fluorescent signals during amplification and therefore allows for highly accurate quantification of RT-PCR products by using a kinetic rather than an endpoint approach. The ratios of hTARPP cDNA from the

temporal cortex and frontal cortex, and from the hippocampus and frontal cortex, respectively, were determined (relative quantification).

First, a standard curve was generated to determine the efficiency of the PCR with specific primers for the gene coding for hTARPP:

5'-ACAGCCAATCATGCTACCTAACC-3' and

5'-CAGTAAACAGGCATTCCAGTGG-3'.

PCR amplification (95 °C and 1 sec, 56 °C and 5 sec, and 72 °C and 5 sec) was performed in a volume of 20 µl containing Lightcycler-FastStart DNA Master SYBR Green I mix (containing FastStart Taq DNA polymerase, reaction buffer, dNTP mix with dUTP instead of dTTP, SYBR Green I dye, and 1 mM $MgCl_2$; Roche), 0.5 µM primers, 2 µl of a cDNA dilution series (final concentration of 40, 20, 10, 5, 1 and 0.5 ng human total brain cDNA; Clontech) and depending on the primers used, additional 3 mM $MgCl_2$. Melting curve analysis revealed a single peak at approximately 84.5°C with no visible primer dimers. Quality and size of the PCR product were determined with the DNA LabChip system (Agilent 2100 Bioanalyzer, Agilent Technologies). A single peak at the expected size of 69 bp for hTARPP was observed in the electropherogram of the sample.

In an analogous manner, the PCR protocol was applied to determine the PCR efficiency of a set of reference genes which were selected as a reference standard for quantification. In the present invention, the mean value of five such reference genes was determined: (1) cyclophilin B, using the specific primers 5'-ACTGAAGCACTACGGGCCTG-3' and 5'-AGCCGTTGGTGTCTTTGCC-3' except for $MgCl_2$ (an additional 1 mM was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 87 °C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band of the expected size (62 bp). (2) Ribosomal protein S9 (RPS9), using the specific primers 5'-GGTCAAATTTACCCTGGCCA-3' and 5'- TCTCATCAAGCGTCAGCAGTTC-3' (exception: additional 1 mM $MgCl_2$ was added instead of 3 mM). Melting curve analysis revealed a single peak at approximately 85°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (62 bp). (3) beta-actin, using the specific primers 5'-TGGAACGGTGAAGGTGACA-3' and 5'-GGCAAGGGACTTCCTGTAA-3'. Melting curve analysis revealed a single peak at approximately 87°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (12 bp). (4) GAPDH, using the specific primers 5'-CGTCATGGGTG-TGAACCATG-3' and 5'-GCTAAGCAGTTGGTGGTGCAG-3'. Melting curve analysis

revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (81 bp). (5) Transferrin receptor TRR, using the specific primers 5'-GTCGCTGGTCAGTTCGTGATT-3' and 5'-AGCAGTTGG-CTGTTGTACCTCTC-3'. Melting curve analysis revealed a single peak at approximately 83°C with no visible primer dimers. Agarose gel analysis of the PCR product showed one single band with the expected size (80 bp).

For calculation of the values, first the logarithm of the cDNA concentration was plotted against the threshold cycle number C_t for the gene coding for hTARPP and the five reference standard genes. The slopes and the intercepts of the standard curves (i.e. linear regressions) were calculated for all genes. In a second step, cDNAs from frontal cortex and temporal cortex, and from hippocampus and frontal cortex, respectively, were analyzed in parallel and normalized to cyclophilin B. The C_t values were measured and converted to ng total brain cDNA using the corresponding standard curves:

$$10 \text{ ^ } ((C_t \text{ value - intercept}) / \text{slope}) \qquad \text{[ng total brain cDNA]}$$

The values for temporal cortex and frontal cortex cDNAs of hTARPP, and the values for hippocampus and frontal cortex cDNAs of hTARPP, respectively, were normalized to cyclophilin B, and the ratio was calculated using the following formula:

$$\text{Ratio} = \frac{\text{hTARPP temporal [ng] / cyclophilin B temporal [ng]}}{\text{hTARPP frontal [ng] / cyclophilin B frontal [ng]}}$$

$$\text{Ratio} = \frac{\text{hTARPP hippocampus [ng] / cyclophilin B hippocampus [ng]}}{\text{hTARPP frontal [ng] / cyclophilin B frontal [ng]}}$$

In a third step, the set of reference standard genes was analyzed in parallel to determine the mean average value of the temporal to frontal ratios, and of the hippocampal to frontal ratios, respectively, of expression levels of the reference standard genes for each individual brain sample. As cyclophilin B was analyzed in

step 2 and step 3, and the ratio from one gene to another gene remained constant in different runs, it was possible to normalize the values for hTARPP to the mean average value of the set of reference standard genes instead of normalizing to one single gene alone. The calculation was performed by dividing the ratios shown above by the deviation of cyclophilin B from the mean value of all housekeeping genes. The results of such quantitative RT-PCR analysis for hTARPP are shown in Figure 2 and 3.

(v) Sequence Analysis

Searching the EST database of the GenBank database for sequence similarities to the identified differentially expressed human cDNA fragment (SEQ ID NO. 4), as stated in the present invention, it was found that SEQ ID NO. 4 is identical to portions of the human EST sequences hms80139 and bg201698 and others (shown in Figure 8). These human ESTs showed homology to mouse (*Mus musculus)* TARPP. Aligning human ESTs in addition to SEQ ID NO. 4, a complete EST cluster representing the hTARPP cDNA, SEQ ID NO. 3, was determined. The amino acid sequence of a large open reading frame, with the potential to encode a protein of 813 amino acid residues was deduced, SEQ ID NO. 1.

(vi) Immunohistochemistry:

For immunofluorescence staining of hTARPP in human brain, frozen sections were prepared from post-mortem pre-central gyrus of a donor person (Cryostat Leica CM3050S) and fixed in acetone at room temperature for 10 min. After washing in PBS, the sections were pre-incubated with blocking buffer (10% normal goat serum, 0.2% Triton X-100 in PBS) for 30min, and then incubated with affinity-purified rabbit anti-hTARPP antisera (1:20-1:40 diluted in blocking buffer, Eurogentec, Herstal, Belgium, custom-made) overnight at 4°C. After rinsing three times in 0.1% Triton X-100/PBS, the sections were incubated with FITC-conjugated goat anti-rabbit IgG (1:150 diluted in 1% BSA/PBS) for 2 hours at room temperature, and then again washed in PBS. Staining of the nuclei was performed by incubation of the sections with 5μM DAPI in PBS for 3min (blue signal). In order to block the autofluoresence of lipofuscin in human brain, the sections were treated with 1% Sudan Black B in 70% ethanol for 2-10 min at room temperature and sequentially dipped in 70% ethanol, destilled water, and PBS. The sections were coverslipped by 'Vectrashield mounting medium' (Vector Laboratories, Burlingame, CA) and observed under an

inverted microscope (IX81, Olympus Optical). The digital images were captured with the appropriate software (AnalySiS, Olympus Optical).